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Original Article

Impacts of Individual Animal Response to Heat and Handling Stresses on *Escherichia coli* and *E. coli* O157:H7 Fecal Shedding by Feedlot Cattle

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Abstract

The reduction of foodborne pathogens in cattle destined for human consumption will require knowledge of the factors that impact the carriage and shedding of these organisms. The objective of this work was to investigate the effects of heat and handling stress levels on the fecal shedding of *Escherichia coli* O157:H7 and generic *E. coli* by feedlot cattle. In year 1, 128 feedlot heifers were evaluated for heat tolerance five times per week during the 84-day finishing period from May through August. Heat stress measurements included respiration rate, panting score, and visual assessments. In year 2, panting scores were taken for a group of 256 finishing feedlot heifers on days in July and August for which the temperature humidity index (THI) was predicted to be in the "emergency" category (THI $\geq$ 84). For both years, animals were weighed and temperament scored to assess handling stress on a 28-day schedule. At the same time, rectal fecal samples were collected from each animal individually. The presence and concentrations of *E. coli* O157:H7 and concentrations of generic *E. coli* in feces were determined. There were no clear trends between the heat stress levels or temperament scores (as an indicator of response to handling) with either fecal generic *E. coli* concentrations or *E. coli* O157:H7 concentrations or prevalence in feces, indicating that neither heat nor handling stress contributes to the food safety risk associated with *E. coli* O157:H7-positive cattle.

Introduction

Escherichia coli O157:H7 causes an estimated 73,480 annual cases of human illness, causing stomach cramps, diarrhea, and vomiting; severe cases may result in hospitalization and even death (Mead *et al.*, 1999). Foodborne illness caused by this pathogen is frequently linked to the consumption of undercooked ground beef, unpasteurized milk, or produce (CDC, 1993; Rangel *et al.*, 2005). Cattle are recognized as an important reservoir of *E. coli* O157:H7. This organism colonizes the bovine gastrointestinal tract and is shed in feces, which can then contaminate the production environment and the animals' hides, potentially resulting in carcass contamination at harvest (Barkocy-Gallagher *et al.*, 2003). Both manure or runoff from livestock feeding operations, manure storage, or manure-amended fields may contaminate food or feed crops, and surface or groundwaters, further increasing the risk for water- or foodborne illness by *E. coli* O157:H7 (Besser *et al.*, 1993; Jackson *et al.*, 1998; O'Connor, 2002; Johnson *et al.*, 2003). Prevalence of *E. coli* O157:H7 shedding by cattle can vary greatly, but most typically is higher during the warmer months (APHIS, 2001; Barkocy-Gallagher *et al.*, 2003; Callaway *et al.*, 2003). Despite the intensive research focus on its preharvest reduction, we do not yet fully understand the factors that may affect either the prevalence of this pathogen in cattle or the number of *E. coli* O157:H7 shed in feces.

Numerous environmental stressors have been identified that may impact infection, prevalence, and/or concentrations of pathogens in livestock, including transport, weaning, relocation, social disruption, crowding, confinement, and dietary changes. With regard to *E. coli* O157:H7, cattle stress (in terms of movement and weaning) was a risk factor for the presence of high-level shedders of *E. coli* O157 on Scottish farms (Chase-Topping *et al.*, 2007). Herriott *et al.* (1998) also reported that abrupt weaning was associated with a higher prevalence of *E. coli* O157 in calves. Bach *et al.* (2004) found that long-haul transport and a lack of preconditioning increased fecal shedding of *E. coli* O157:H7 and *E. coli* in range calves, and concluded that the stresses of weaning, transport, and

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relocation likely increased their susceptibility to infection. Feed withdrawal or fasting associated with transportation of cattle may also increase shedding of *E. coli* O157:H7 (Cray *et al.*, 1998; Buchko *et al.*, 2000). Transportation to and lairage at beef processing facilities have been associated with increased prevalence of *E. coli* O157:H7 on cattle hides, but this increase also may have been due to exposure and contact to contaminated fecal material present in the transport truck and lairage environments (Arthur *et al.*, 2007; Dewell *et al.*, 2008), in addition to increased fecal shedding (Bach *et al.*, 2004). Schuehle Pfeiffer *et al.* (2009) reported that transportation did not increase fecal shedding of *E. coli* O157:H7 by feedlot cattle, but observed that cattle with calmer temperaments, as compared to cattle with excitable temperaments, can have higher fecal prevalence of this pathogen. Edrington *et al.* (2004) did not observe a clear effect of heat stress on the shedding of *E. coli* O157:H7 or *Salmonella* in lactating dairy cattle. However, the hypothesis remains intriguing because of the increase in prevalence of fecal shedding of *E. coli* O157:H7 by cattle during the warmer months (APHIS, 2001; Barkocy-Gallagher *et al.*, 2003).

It is generally accepted that stress suppresses components of the immune system, leading to an increased susceptibility to infection should the pathogen be present (Salak-Johnson and McGlone, 2007). While causing disease in humans, *E. coli* O157:H7 is not considered to be a pathogen of cattle, and it commonly occurs in cattle without apparent effects on performance (Berry *et al.*, 2006). However, cattle do elicit an immune response to *E. coli* O157:H7, and vaccines have been developed and tested as a preharvest intervention to reduce this pathogen in cattle (Peterson *et al.*, 2007a, 2007b). The objectives of this work were to determine the effects of heat stress and handling stress (as measured by temperament score) on the prevalence and concentration of *E. coli* O157:H7 and the concentration of generic *E. coli* shed in feces of feedlot cattle during the finishing period.

Materials and Methods

All animal procedures were reviewed and approved by the U.S. Meat Animal Research Center Animal Care and Use Committee. The experiments were carried out over a 2-year period. During year 1, the study concentrated on smaller groups of animals, and intensive stress measurements were made on the animals over the 84-day finishing period. The measurements made during the second year were on large groups of animals in a setting that more closely mimicked the beef production industry, with fewer stress measurements made, that were concentrated on hot days.

Year 1

One hundred and twenty-eight finishing feedlot heifers of four breeds (Angus, Charolais, MARC I [1/4 Charolais, 1/4 Braunvieh, 1/4 Limousin, 1/8 Angus, and 1/8 Hereford], and MARC III composite breed cattle [1/4 Pinzgauer, 1/4 Red Poll, 1/4 Hereford, and 1/4 Angus]) were selected from the U.S. Meat Animal Research Center's (USMARC) population. The heifers initially weighing 401.7 ± 35.4 kg were assigned to one of 16 pens by breed, weight, and preexperiment *E. coli* O157:H7 shedding status (each pen having a total of eight heifers, two of each breed). Heifers were implanted with Synovex-H (200 mg testosterone propionate and 20 mg estradiol benzoate) before the study began. The animals were allowed *ad libitum* access to a standard feedlot diet fed twice daily before 0800 h and after 1300 h, and were given free access to water.

The pens allowed 19.0 m^2/animal of pen space (Harner and Murphy, 1998). Of the 16 pens, 8 pens had one half of the total pen space under shade (9.5 m^2/animal of available shade). The pens had the identical configuration with the exception of the shade. Shade was provided using a noninsulated galvanized steel roof. The gabled roof had eave heights of 3.7 m and a peak height of 6.1 m. The feed bunk and the water tank were both located under the shade.

The heifers were evaluated for heat tolerance by measurements of respiration rate and panting score taken 5 days per week during the 84-day finishing period from May through August (Brown-Brandl *et al.*, 2006). The animals were preconditioned to observers for a period of 2 weeks before the stress measurements were recorded. The 128 animals were assigned to groups 1 or 2 for respiration rate and panting score observations. The groups were observed on alternating days Monday through Friday after 1400 h, such that animals in one group were evaluated 2 days in 1 week and 3 days in the next week, and vice versa. Two observers manually took respiration rates using a stopwatch to time 10 flank movements. At the same time, a 0 to 4 visual assessment of an animal's level of heat stress, panting scores, were assigned (0 = not stressed, normal respiration; 1 = elevated respiration; 2 = presence of drool; 3 = open mouth breathing; 4 = open mouth breathing accompanied by a protruding tongue). The respiration rate and panting score values recorded by the two observers were averaged and reported as a single value. Observers also recorded animal ID and pen number at the same time as the stress measurements were taken.

Heifers were weighed and temperament scored on a 28-day schedule. Temperament was scored by two observers using the method described by Voisinet *et al.* (1997), in which a 1 to 5 score is assigned to individual animals based on their behavior while confined in the chute. The two observers' temperament scores were averaged and reported as a single score. A fecal grab sample was collected from each animal immediately after the collection of temperament score data.

Year 2

Two hundred and fifty-six finishing feedlot heifers from a group of different breeds and cross-breeds from the USMARC cattle herd, initially weighing 420.4 ± 51.5 kg, were assigned to one of eight pens on the basis of weight and preexperiment *E. coli* O157:H7 shedding status. Heifers were implanted with Synovex-H before the study began. The animals were fed a standard feedlot diet twice daily before 0800 h and after 1300 h, and were given free access to water throughout the experiment. Animals were provided with 36.9 m^2/animal of pen space (Harner and Murphy, 1998).

Panting scores were taken on days in July and August for which the temperature humidity index (THI) was predicted to be in the "emergency" category (THI $\geq$ 84). Panting scores were assessed on all animals in a pen after 1400 h. The observers were careful not to disturb the animals during the observation.

The second year protocol was similar to that of year 1, with heifers weighed on a 28-day schedule (Brown-Brandl *et al.*, 2006). Cattle were temperament scored at the July weighing

by one observer. At both the July and August weighing, a fecal grab sample was collected from each animal.

Weather data

Hourly weather data were downloaded from the High Plains Climate Center (HPCC). The weather station is located 3.2 km east and 1.3 km north of the USMARC feedlot. Data retrieved from the HPCC included temperature and relative humidity. THI was calculated using HPCC data and Equation 1, where t_{db} is dry-bulb temperature (°C) and RH is relative humidity (%).

$$THI = 0.8t_{db} + \frac{RH(t_{db} - 14.4)}{100} + 46.4 \quad (1)$$

Microbial analyses

Rectal fecal samples were obtained directly from each animal, using a clean shoulder-length glove for each sample. Samples were returned to the laboratory for immediate processing. Both the presence and concentration of *E. coli* O157:H7 and the concentration of generic *E. coli* in each fecal sample were determined. Ten grams of feces was measured into a sterile filtered sample bag (Nasco, Ft. Atkinson, WI), and 90 mL of tryptic soy broth (Becton, Dickinson and Company, Sparks, MD) containing phosphate buffer was added. The bag contents were mixed by massaging the bag, and a 1 to 2 mL volume was removed to a sterile tube for use in determining bacterial populations. This volume was diluted further in buffered peptone water as necessary, and 50 μL volumes were spiral-plated using an Autoplate 4000 spiral plater (Spiral Biotech, Norwood, MA) onto each of CHROMagar O157 (DRG International, Mountainside, NJ) containing novobiocin (5 mg/L) and potassium tellurite (1 mg/L; ntCHROMagar O157) for the determination of *E. coli* O157:H7 concentrations, and CHROMagar ECC (DRG International) for the determination of generic *E. coli* concentrations. The ntCHROMagar O157 plates were incubated 42°C for 20 to 24 h, and the CHROMagar ECC plates were incubated at 37°C for 20 to 24 h, before examination and enumeration of typical colonies. Suspect *E. coli* O157:H7 colonies were tested for agglutination using the Dryspot *E. coli* O157 latex test (Oxoid, Basingstoke, England). Isolate identities were confirmed by multiplex PCR for genes specific for enterohemorrhagic *E. coli* and *E. coli* O157:H7 according to the method of Hu *et al.* (1999).

The remaining diluted samples were subjected to enrichment and immunomagnetic separation as described in Barkocy-Gallagher *et al.* (2002, 2005). After immunomagnetic separation, 50 μL volumes of the concentrated bead suspensions were plated onto each of ntCHROMagar O157 and sorbitol MacConkey agar (Difco, Becton, Dickinson and Company) containing cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L). Plates were incubated at 37°C for 20 to 24 h and examined for suspect *E. coli* O157:H7 colonies, which were tested for agglutination and confirmed by PCR as described above.

Statistical analyses

Two different analyses were performed. The effects of stress level (handling and heat stress) on the fecal concentrations of generic *E. coli* and *E. coli* O157:H7 were analyzed using a linear regression analysis within MS Excel, with individual animal as the experimental unit. For this analysis, the temperament scores, panting scores, and respiration rates (year 1 only) were used to describe the stress level. The effects of stress level (handling and heat stress levels) on the prevalence of *E. coli* O157:H7 were analyzed using the Fisher Exact analysis (Uitenbroek, 2000). Animals were classified into low, medium, and high stress categories for both heat stress and handling stress (temperament score, described below) for the prevalence data analysis. The number of *E. coli* O157:H7-positive animals in each category was summed.

Temperament scores were used to classify the levels of handling stress that individual animals experienced while confined in the chute. Animals with temperament scores of 1 were classified in the low handling stress category, animals with a temperament score of greater than 1 but less than 2.5 were classified in the medium handling stress category, while animals with a temperament score of greater than 2.5 were classified in the high handling stress category.

Respiration rates (year 1 only) and panting scores were both used to classify animals' heat stress levels. Data were used only from days with maximum THI $\geq$ 80.5 [the "mid danger" and "emergency" categories (LCI, 1970; Hahn, 1999)], because differences in cattle heat tolerance typically are not discernible when THI $<$ 80.5. Panting scores and respiration rates on these days were averaged over the given 28-day sampling period. Animals with an average panting score less than or equal to 1 were classified in the low stress category, animals with an average panting score greater than 1 but less than 1.75 were classified in the medium stress category, and animals with an average panting score greater than 1.75 were classified in the high stress category. Respiration rates were also analyzed for year 1. Animals with an average respiration rate less than 90 breathes/min were classified in the low stress category, animals with an average respiration rate greater than 90 but less than 110 were classified in the medium category, and animals with an average respiration rate of greater than 110 were classified in the high stress category.

Results and Discussion

The development of successful preharvest interventions to reduce zoonotic pathogens in livestock requires knowledge of the factors that affect the prevalence and shedding of these pathogens. Animal stress is one factor that has received attention as a factor that may promote colonization and affect the concentrations of pathogens shed by animals (Edrington *et al.*, 2004, Vlisidou *et al.*, 2004). In this work, we examined the impact of heat and handling stresses on the fecal carriage and shedding of *E. coli* O157:H7 in feedlot cattle. The fecal concentrations of *E. coli* O157:H7 (when present) typically are low, and below the threshold of detection of most enumeration procedures (Brichta-Harhay *et al.*, 2007). As *E. coli* O157:H7 and other pathogenic *E. coli* are a subset of the species *E. coli*, one can anticipate that their response to stress would be similar to that of the larger group. For these reasons, we also determined the impact of cattle stress on the fecal concentrations of indigenous, generic *E. coli*.

The prevalence of *E. coli* O157:H7 decreased ($p < 0.0001$) during year 1, from 57.0% (73/128) animals positive in May to only 3.9% (5/128) animals positive in August (32.8% and

Table 1. Average Panting Score (PS) and Respiration Rate (RR) of the Feedlot Cattle, and Average and Maximum Temperature Humidity Index (THI) Measured on Days on Which the Maximum THI Was ≥ 80.5, During Years 1 and 2[a]

Year and sample period (fecal sample collection date)	*Heat stress assessment dates*[b]	*Number of animals sampled*	*Group*[b]	*Average THI (SD)*	*Maximum THI*	*Average panting score*[c] *(SD)*	*Average respiration rate (SD)*
Year 1							
0 (5/15)							
1 (6/12)	6/9	64	2	73.8 (5.7)	80.5	0.77 (1.01)	103.4 (17.7)
2 (7/10)	6/20	64	2	75.2 (4.2)	82.2	1.17 (1.13)	102.3 (19.9)
	6/30	64	1	71.6 (7.2)	80.5	1.10 (0.83)	107.4 (19.6)
3 (8/7)	7/13	64	2	74.6 (6.1)	83.1	1.23 (0.62)	125.2 (19.5)
	7/14	64	1	74.1 (7.5)	82.8	1.29 (0.62)	131.7 (20.6)
	7/17	64	2	74.4 (5.1)	81.4	1.09 (0.73)	120.6 (20.2)
	7/18	64	1	72.4 (6.9)	81.0	0.77 (0.66)	110.8 (18.9)
	7/19	64	2	77.1 (4.9)	83.5	1.54 (0.61)	126.2 (19.8)
	7/25	64	2	74.7 (5.4)	82.6	1.23 (0.84)	100.7 (19.9)
	7/26	64	1	74.6 (5.7)	81.4	0.80 (0.71)	108.7 (18.9)
	7/31	64	2	77.1 (3.8)	81.9	0.71 (0.75)	99.8 (21.0)
	8/1	64	1	75.9 (3.8)	82.2	1.26 (0.64)	96.5 (18.6)
Year 2							
1 (7/16–7/17)	7/7	250	NA	73.8 (5.6)	81.3	1.33 (0.86)	ND
	7/8	251	NA	73.9 (6.4)	82.1	1.62 (0.72)	ND
2 (8/6–8/7)	7/17	225	NA	73.7 (5.6)	81.2	1.15 (0.85)	ND
	7/18	249	NA	75.3 (4.8)	82.1	1.00 (0.74)	ND
	7/23	222	NA	74.3 (5.0)	81.5	1.50 (0.56)	ND

[a]In year 1, PS and RR (breathes/min) were assessed on animals daily after 1400 h. In year 2, PS was assessed on animals after 1400 h, on days for which THI was predicted to be in the "emergency" category (THI ≥ 84).

[b]As described in the Materials and Methods section, the 128 cattle used in year 1 were divided into two groups of 64, and RR and PS were assessed and recorded on one group of animals per day on an alternating schedule.

[c]PS is a visual assessment of an animal's heat stress level: 0 = not stressed, normal respiration; 1 = elevated respiration; 2 = presence of drool; 3 = open mouth breathing; 4 = open mouth breathing accompanied by a protruding tongue.

NA, not applicable; ND, not determined.

7.8% of the 128 animals were positive for *E. coli* O157:H7 in June and July, respectively). This decrease in prevalence during the summer months is in contrast with numerous previous observations of increased prevalence of this pathogen during these warmer months (APHIS, 2001; Barkocy-Gallagher *et al.*, 2003; Callaway *et al.*, 2003). During year 2, the prevalence of *E. coli* O157:H7 increased ($p < 0.0001$) during the sampling period; 15.2% (39/256) animals were positive in July, and 28.9% (74/256) animals tested positive in August. In year 1, animals were penned in groups of eight per pen, while in year 2, the animals were penned in larger groups of 32 per pen. Also, the animals were provided 19.0 m^2 per animal during year 1, almost half the space allowed per animal during year 2 (36.9 m^2). While this may be due in part to year-to-year variation, it is interesting to speculate that the number of animals in a given pen may have a larger effect on *E. coli* O157:H7 prevalence than the concentration of animals in that pen. A greater number of animals in a pen may increase the probability that one or more animals present are shedding high enough numbers to maintain the carriage rate of this pathogen among the animals in the pen. The presence of an animal shedding high concentrations of *E. coli* O157:H7 ($>10^3$ or 10^4 CFU/g of feces) on a given farm has been associated with a higher prevalence of shedding of this organism on that farm (Matthews *et al.*, 2006; Chase-Topping *et al.*, 2007).

The minimum threshold of detection of the procedure employed to enumerate *E. coli* O157:H7 in feces is 2.30 $\log_{10}$ CFU/g. *E. coli* O157:H7 concentrations typically are below detectable levels, but concentrations ranging from 2.00 to 6.00 $\log_{10}$ CFU/g of feces have been reported (Omisakin *et al.*, 2003; Brichta-Harhay *et al.*, 2007). We observed similar concentrations. Among the *E. coli* O157:H7-positive fecal samples found in year 1, 39.7%, 21.4%, 40%, and 40% (29/73, 9/42, 4/10, and 2/5) in May, June, July, and August, respectively, had *E. coli* O157:H7 cell concentrations that were enumerable by the spiral-plating method. Concentrations of *E. coli* O157:H7 among these enumerable samples ranged from 2.30 to 6.27 $\log_{10}$ CFU/g of feces. In year 2, 14 of the 39 positive samples in July (35.9%) and 35 of the 74 positive samples in August (47.3%) had enumerable levels of *E. coli* O157:H7, with concentrations ranging from 2.30 to 6.21 $\log_{10}$ CFU/g of feces.

Heat stress

During year 1, there were a total of 19 days for which the maximum THI exceeded 80.5. Animal stress measurements were taken on 12 of those days: 1 day before the June sampling day, 2 days before the July sampling day, and a total of 9 days before the August sampling day. Heat stress data were collected on group 1 a total of 5 days and on group 2 a total of 7 days. Year 2 was considerably cooler than year 1. During year 2, there were 8 days that maximum THI exceeded a THI of 80.5 or greater. Heat stress data were collected a total of 5 days, 2 days before the July sampling day and 3 days before the August sampling day. Sampling dates, average and

Table 2. Regression Analysis Between Cattle Heat or Handling Stress Level Measurements and Generic *Escherichia coli* or *E. coli* O157:H7 Concentrations in Their Feces[a]

Bacterial population, year, and stress level measurement	*Intercept (SE)*	*Coefficient (SE)*	p-*Value*	R^2
Generic *E. coli*				
Year 1				
RR (breathes/min)	6.98 (0.23)	−0.0011 (0.002)	0.5956	0.0009
PS[b]	6.81 (0.05)	0.049 (0.044)	0.2615	0.004
Temperament score[c]	6.93 (0.09)	−0.202 (0.05)	<0.0001	0.033
Year 2				
PS	6.21 (0.06)	0.056 (0.043)	0.192	0.058
Temperament score[d] (vs. *E. coli* concentrations on 7/16–7/17)	6.51 (0.10)	0.022 (0.06)	0.7207	0.0005
Temperament score (vs. *E. coli* concentrations on 8/6–8/7)	6.00 (0.09)	0.014 (0.06)	0.7997	0.0003
E. coli O157:H7—years 1 and 2[e]				
PS	3.63 (0.292)	−0.095 (0.220)	0.6695	0.0031
Temperament score	3.56 (0.345)	0.038 (0.199)	0.8480	0.0004

[a]In year 1, PS and RR were assessed on animals daily after 1400 h. In year 2, PS was assessed on animals after 1400 h, on days for which THI was predicted to be in the "emergency" category (THI ≥ 84). Temperament was scored and fecal concentrations of generic *E. coli* were determined for each animal every 28 days.

[b]PS is a visual assessment of an animal's heat stress level: 0 = not stressed, normal respiration; 1 = elevated respiration; 2 = presence of drool; 3 = open mouth breathing; 4 = open mouth breathing accompanied by a protruding tongue.

[c]Temperament was scored using the method described by Voisinet *et al.* (1997), in which a 1 to 5 score is assigned to individual animals based on their behavior while confined in the chute.

[d]In year 2, temperament was scored at the 7/16–7/17 sampling day only. This temperament scored is analyzed against generic *E. coli* fecal concentrations on 7/16–7/17 and 8/6–8/7.

[e]Because of the limited number of fecal samples with enumerable *E. coli* O157:H7 ($n = 93$), data for years 1 and 2 were pooled for analysis.

RR, respiration rate; PS, panting score.

maximum THI, average panting score, and average respiration rate are presented in Table 1.

The analyses of the data collected in both years 1 and 2 show little evidence of an association between heat stress levels (as measured by respiration rate or panting score) and fecal generic *E. coli* concentrations (Table 2). Figures 1 and 2 illustrate the lack of relationship between heat stress and concentrations of generic *E. coli*. Similarly, *E. coli* O157:H7 concentrations in cattle feces were not affected by heat stress level as measured by panting score (Table 2). Respiration rates were recorded in year 1 only. Because of the decrease in *E. coli* O157:H7 prevalence during the study period of year 1, there were only 12 animals with fecal samples containing enumerable *E. coli* O157:H7 for which respiration rates were recorded; because of this limited number of observations, we did not analyze heat stress level as measured by respiration rate versus *E. coli* O157:H7 fecal concentration.

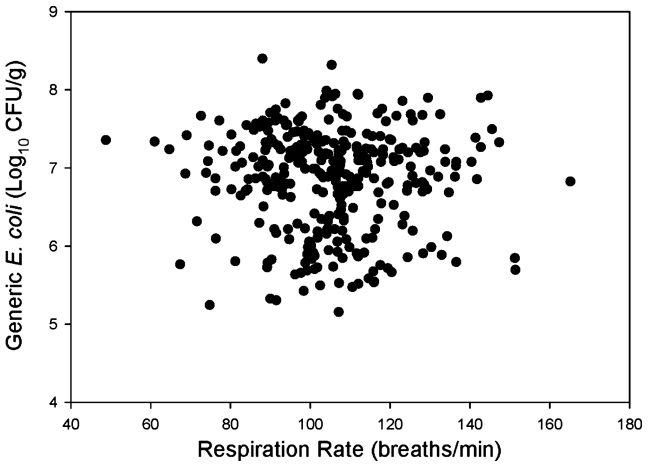

FIG. 1. Impact of heat stress level on generic *Escherichia coli* concentrations in the feces of feedlot cattle, as measured by average respiration rates on days for which the maximum temperature humidity index was greater than or equal to 80.5. Data were collected during the summer of year 1.

For the purposes of analyzing the *E. coli* O157:H7 prevalence data, the animals were categorized into stress categories as described in the Materials and Methods. The number of animals in a specific category for each sampling period and the number of animals found positive for *E. coli* O157:H7 are shown in Table 3. Only one significant result was found: in sample period 2 (6/12 through 7/10) of year 1, the respiration rate-medium stress group had significantly reduced pathogen shedding as compared to either the respiration rate-high or respiration rate-low stress groups. However, there were no differences between panting score stress groups for this same period. Further, there were no consistent trends among sampling periods or across years. This analysis may also have been complicated by the reduction in prevalence during the year 1 sampling period; *E. coli* O157:H7 prevalence decreased from 32.8% to 7.8% from June to July of that year. Thus, this result may not be meaningful. As noted previously, the fecal prevalence of *E. coli* O157:H7 is seasonal, and the association of higher fecal prevalence with the warmer summer and fall months has led to hypotheses regarding the effect of temperature, including cattle heat stress, on the incidence of this pathogen. However, data from this study and others (Fitzgerald *et al.*, 2003; Edrington *et al.*, 2004) do not support the hypothesis that heat stress of cattle impacts the shedding of *E. coli* O157:H7.

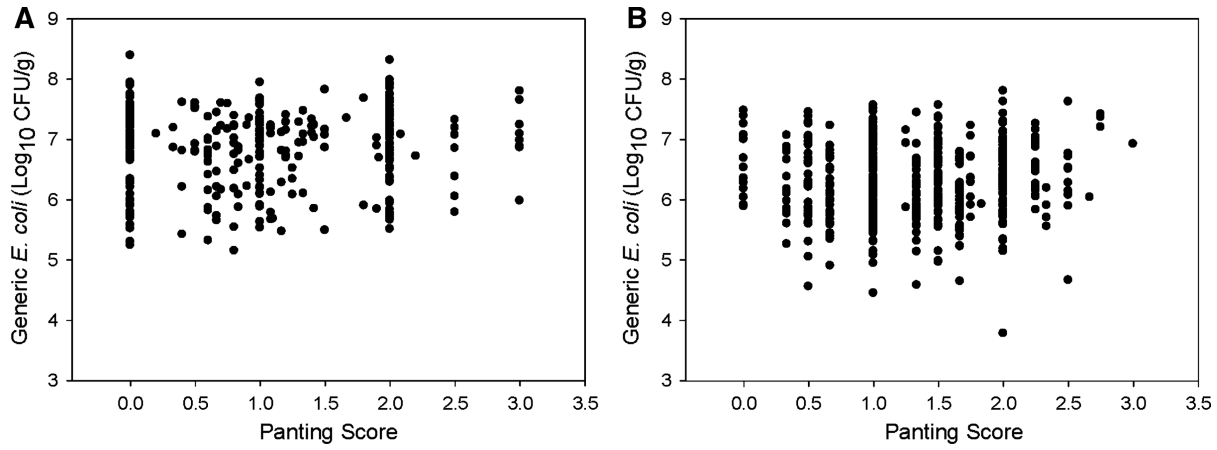

FIG. 2. Impact of heat stress level on generic *Escherichia coli* concentrations in the feces of feedlot cattle, as measured by panting scores on days for which the maximum temperature humidity index was greater than or equal to 80.5. (**A**) Year 1. (**B**) Year 2.

In addition to heat stress level in year 1, the effects of providing shade were also tested. Shade has been shown to reduce overall impact of heat on the animals by reducing the solar load (Bond *et al.*, 1967; Blackshaw and Blackshaw, 1994; Busby and Loy, 1996; Brown-Brandl *et al.*, 2005). If heat stress levels affect *E. coli* O157:H7 shedding by cattle, then providing a shaded environment might also affect shedding, but one common problem observed with providing shade is the increase in soil moistures in the shade. Increase in soil moisture may provide an environment that is more favorable for the survival of the pathogens or other fecal bacteria. However, results of this study (Table 3) suggest that providing shade to feedlot cattle has no impact on the prevalence of *E. coli* O157:H7 during the summer months ($p > 0.2$). Further, neither the prevalence of *E. coli* O157:H7 ($p = 0.59$) nor the concentrations of generic *E. coli* ($p = 0.60$) in feedlot surface material from shaded and nonshaded regions of the feedlot pens were different (data not shown). This result is similar to the results of Morrow *et al.* (2005), who reported no change in the incidence of *E. coli* O157:H7 or *Salmonella* that were shed in feces when water sprinkling was used to alleviate dust and cool feedlot cattle. While sprinkling cattle is a different management strategy than is shade, it has similar effects with regard to lowering the impact of hot weather on the stress level of the animals and increasing soil moisture.

Handling stress

Cattle temperament can be assessed by several methods, including chute scores, exit velocities, and pen scores (Curley *et al.*, 2006). All three of these methods are correlated with each other ($R^2 = 0.35$) (Curley *et al.*, 2006). Chute scores, utilized in this study, are a subjective visual assessment in which

Table 3. The Impact of Heat Stress, as Measured by Average Respiration Rate (RR; Breathes/Min) or Average Panting Score (PS), and the Effects of Providing Shade on the Fecal Prevalence of *Escherichia coli* O157:H7 in Cattle[a]

Year, sample period, and heat stress measure	E. coli *O157:H7-positive samples/total samples*				
	Low stress	*Medium stress*	*High stress*	*No shade*	*Shade*
Year 1					
Sample period 1–RR[b]	8/17	7/23	3/24	20/64	22/64
Sample period 1–PS[c]	12/42	—	6/22		
Sample period 2–RR	3/28[d]	0/56[e]	7/44[d]	4/64	6/64
Sample period 2–PS	4/73	0/1	6/54		
Sample period 3–RR	0/10	4/75	1/43	4/64	1/64
Sample period 3–PS	2/78	2/41	1/9		
Year 2					
Sample period 1–PS	19/95	5/51	15/110	NA	NA
Sample period 2–PS	33/129	26/93	15/33	NA	NA

[a]In year 1, PS and RR were assessed on animals daily after 1400 h. In year 2, PS was assessed on animals after 1400 h, on days for which THI was predicted to be in the "emergency" category (THI $\geq$ 84).

[b]RR values were used to assign animals to heat stress categories by the following protocol: low stress, average RR $<$ 90; medium stress, 90 $<$ average RR $<$ 110; high stress, average RR $\geq$ 110.

[c]PS is a visual assessment of an animal's heat stress level: 0 = not stressed, normal respiration; 1 = elevated respiration; 2 = presence of drool; 3 = open mouth breathing; 4 = open mouth breathing accompanied by a protruding tongue. PS was used to assign animals to categories by the following protocol: low stress, average PS = 1; medium stress, 1 $<$ average PS $<$ 1.75; high stress, average PS $\geq$ 1.75.

[d,e]Different letters in the same row indicate significant differences ($p \leq 0.05$).

NA, not applicable.

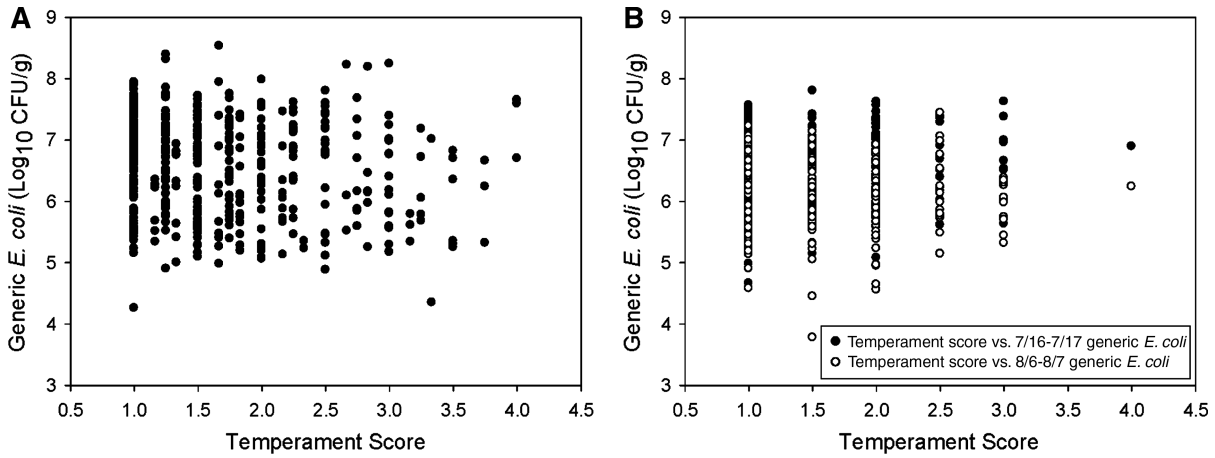

FIG. 3. Impact of handling stress level, as measured by temperament score, on generic *Escherichia coli* concentrations in the feces of feedlot cattle. (**A**) Year 1. (**B**) Year 2. In year 2, temperament was scored at the 7/16–7/17 sampling only; shown are temperament scores versus generic *E. coli* concentrations at the 7/16–7/17 sampling (closed symbols) and the 8/6–8/7 sampling (open symbols).

a 1 to 5 score is assigned to the animal based on its behavior while confined in the chute. This method is based on the industry standard for temperament scoring (Voisinet *et al.*, 1997), and also is associated with the behavior of the animal in the pen (King *et al.*, 2006). Therefore, the chute scores assigned to individual animals would be associated with the acute stress of being confined in the chute for weighing and data collection, as well as the stress associated with occasional working events or daily checking by feedlot personnel.

There was little or no association between response to handling (as measured by temperament score) and generic *E. coli* or *E. coli* O157:H7 concentrations in feces (Table 2). A significant effect of temperament score with generic *E. coli* concentration was found in the experiment conducted in year 1. However, that the correlation coefficient was very low ($R^2 = 0.033$) and that the effect was not found in the experiment conducted in the second year lead to the conclusion that the significance was arbitrary. Figure 3 illustrates the lack of correlation between temperament score and concentrations of fecal generic *E. coli*.

For the purpose of analyzing the *E. coli* O157:H7 prevalence data, temperament scores were categorized into low, medium, and high handling stress categories. The only significant effect observed was during July of year 2. A significantly greater proportion of the animals in the medium handling stress category were positive for *E. coli* O157:H7 as compared to animals in the low handling stress category (Table 4). The high handling stress group was not significantly different than the low or the medium categories. The occurrence of only one significant event and a lack of other observable trends suggest a weak or insignificant effect. In their study of the effects of transportation and temperament on *E. coli* O157:H7 fecal

Table 4. The Impact of Cattle Handling Stress, as Measured by Average Temperament Score, on the Fecal Prevalence of *Escherichia coli* O157:H7[a]

	E. coli *O157:H7-positive samples/total samples*		
Year and sample date	*Low stress*	*Medium stress*	*High stress*
Year 1			
5/15	11/16	50/87	12/25
6/12	11/29	26/87	5/12
7/10	7/72	2/51	1/5
8/7	1/5	4/56	0/15
Year 2[b]			
7/16–17 Temperament score vs. 7/16–17 *E. coli* O157:H7 prevalence	12/113[d]	24/118[c]	3/25[c,d]
7/16–17 Temperament score vs. 8/6–7 *E. coli* O157:H7 prevalence	27/113	40/118	7/25

[a]Temperament was scored using the method described by Voisinet *et al.* (1997). Animals with average temperament scores of 1 were classified in the low handling stress category, animals with average temperament scores of greater than 1 but less than 2.5 were classified in the medium handling stress category, while animals with an average temperament score of greater than 2.5 were classified in the high handling stress category. Temperament scores and fecal prevalence of *E. coli* O157:H7 were determined for each animal on a 28-day schedule.

[b]In year 2, temperament was scored only once, on 7/16–7/17 sampling dates. *E. coli* O157:H7 prevalence on both 7/16–7/17 and 8/6–8/7 were used to test the effect of handling stress on prevalence using temperament score collected on 7/16–7/17.

[c,d]Different letters in the same row indicate significant differences ($p \leq 0.05$).

shedding by cattle, Schuehle Pfeiffer *et al.* (2009) observed that a higher proportion of cattle with calm temperaments were positive for the pathogen than cattle with excitable or intermediate temperaments, at some stages of production. However, they also cautioned that the low prevalence of *E. coli* O157:H7 throughout their study may have biased their results. Nevertheless, their results, in combination with our work, indicate a need for additional studies examining cattle temperament and pathogen shedding.

Various environmental and social stresses are generally considered to influence pathogen colonization by altering animal immune function (Salak-Johnson and McGlone, 2007). Further, some *in vitro* studies have indicated that neuroendocrine or immune molecules associated with these stresses may enhance the ability of pathogens, including *E. coli* O157:H7, to grow and/or colonize their hosts. For example, the catecholamine norepinephrine is released into the gastrointestinal tract upon animal stress. *E. coli* has been demonstrated to take up norepinephrine and to respond to norepinephrine with stimulated growth and expression of a number of factors associated with virulence and colonization, including adhesin, Shiga-like toxins, and LEE-encoded proteins (Lyte *et al.*, 1997; Kinney *et al.*, 2000; Sperandio *et al.*, 2003; Bansal *et al.*, 2007; Freestone *et al.*, 2007, 2008). Using a bovine ligated ileal loop model of infection, Vlisidou *et al.* (2004) examined the effect of norepinephrine on the adherence and enteropathogenicity of *E. coli* O157:H7 and found that norepinephrine enhanced adherence of the pathogen to the intestinal mucosa. As another example, the glucocorticoid dexamethasone can induce immunosuppression in cattle and has been widely used to enhance experimental infections for research purposes. Dexamethasone immunosuppression may simulate that which is experienced by cattle as a result of environmental stresses such as handling or transportation (Stoffregen *et al.*, 2004; Sreerama *et al.*, 2008). Dean-Nystrom *et al.* (2008) found that dexamethasone treatment enhanced the susceptibility of weaned calves to *E. coli* O157:H7 colonization and that fecal and intestinal concentrations of the pathogen were higher in dexamethasone-treated calves than in nontreated calves. Regarding the effects of environmental stress (as opposed to chemically induced stress), growth of *E. coli* O157:H7 in implanted intraperitoneal chambers tended to be greater in mice stressed by social conflict when compared to that in nonstressed mice (Dréau *et al.*, 1999).

We did not observe changes or differences in *E. coli* O157:H7 prevalence or *E. coli* populations as a result of differences in handling or heat stress levels of cattle. However, as outlined by Salak-Johnson and McGlone (2007) the relationships between stressors and immunity in livestock are complex, and elucidation awaits greater understanding of these interactions. Many factors may influence livestock response to stress or the results of studies examining these responses, including stressor type, duration of stress (acute versus chronic), sample type, or the time of sample collection relative to the onset of stress (Salak-Johnson and McGlone, 2007). By making multiple stress observations of each animal and averaging the stress response (respiration rates, panting scores, and/or temperament scores), we were quantifying each animal's chronic stress level and its effects on *E. coli* O157:H7 prevalence and generic *E. coli* concentrations. Because the appropriate timing is uncertain for fecal sample collection in relation to stress observations, we collected multiple fecal samples from each animal over the course of the experiments. By designing the experiment and analyzing the data in this way, the questions we attempted to answer were, are cattle that experience higher/lower heat stress (or have excitable/calm temperaments) more likely to be positive/negative for *E. coli* O157:H7, or have higher/lower fecal concentrations of *E. coli*? Neither heat nor handling stress levels of cattle appeared to influence *E. coli* O157:H7 or generic *E. coli* shedding; thus, these stresses do not contribute to the food safety risk associated with *E. coli* O157:H7-positive cattle.

Conclusions

Over a 2-year period, feedlot cattle were individually observed for signs of heat stress on days when the THI was in the "mid-danger" or "emergency" categories (THI > 80.5). The cattle were also temperament scored during the normal 28-day weighing and microbiological sampling schedule. Correlations were tested on individual animal heat and handling stress levels and the concentrations of generic *E. coli* and *E. coli* O157:H7 in their feces. In addition, analyses were conducted to test for differences in prevalence of *E. coli* O157:H7 as a result of experiencing different levels of heat or handling stress. No evidence was found that would suggest a relationship between either handling or heat stress of cattle and generic *E. coli* concentrations or *E. coli* O157:H7 concentrations and prevalence in their feces.

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